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Immunological Resorption in *Botryllus schlosseri* (Tunicata) Chimeras is Characterized by Multilevel Hierarchial Organization of Histocompatibility Alleles. A Speculative Endeavor

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Laboratory studies on chimeras of the tunicate Botryllus schlosseri *revealed the phenomenon of "allogeneic resorption" or "colony resorption," the elimination of one partner in a chimera. A hypothesis outlining the genetic basis of the resorption phenomenon is presented here. It proposes that allogeneic resorption in* Botryllus *is controlled by a polymorphic, ladder-like, tri-level hierarchial system of histocompatibility loci, with codominantly expressed alleles. The relative state of homozygosity at these alleles within each level reflects the state of the hierarchy in the resorption phenomenon. The Fu/HC haplotype of the tunicates serves as the first level, whereas the other two levels are each controlled by specific resorption alleles (Re/HC) that operate only after a response has failed to occur at previous levels. Some morphologically expressed similarities to murine minor histocompatibility loci (Hh-1 and M1s) are discussed.*

Members of the colonial protochordate *Botryllus schlosseri*, a cosmopolitan, encrusting, subtidal species, have the property of forming either a natural transplantation (vascular anastomosis) or a rejection when confronting conspecific colonies. This histocompatibility character is controlled by a single, highly polymorphic Mendelian locus, with multicodominantly expressed alleles (1–3). This locus, termed the tunicate Fu/HC haplotype (4, 5), resembles in some of its properties the vertebrate MHC, the major histocompatibility complex (3). However, the rules for histocompatibility in *Botryllus* differ significantly from the MHC-dependent graft rejection of vertebrates, in which grafts are accepted only between animals that express the same form of each type of histocompatibility antigens. In tunicates, two colonies sharing at least one allele at the Fu/HC locus may fuse, whereas rejection develops only between colonies that share no allelic determinant at this locus.

In many laboratory studies done with several hundred pairs of fused colonies (chimeras), we have shown (4–12) that once colonies have fused, the chimeras do not survive or grow as well as genetically identical subclones that were left separate. Moreover, fusions usually terminate in the unilateral, morphological elimination of all zooid elements of only one partner within each specific chimera, a phenomenon termed "allogeneic resorption" or "colony resorption" (5). Colony resorption appears to be determined genetically, rather than by relative contribution to the chimera; *i.e.*, when multiple ramets from colony A are allowed to fuse with ramets from colony B, the direction of resorption is usually the same. Progenitor zooids of only one interacting genotype in each pairwise combination are the chimeric survivors.

Our two recent publications (4, 13) have provided some insight into the genetic background governing colony resorption. When pairs of colonies were selected genotypically for "one way allorecognition" (*e.g.*, AA *vs.* AB), the Fu/HC homozygotic partners were resorbed preferentially, but not exclusively [(4) and Y. Saito, unpub.]. This result does not follow the rules underlying MHC-related graft rejection in vertebrates, where the differences in the biochemical composition of the MHC molecules in a similar assay will be recognized only by the homozygotic partner, which will destroy the cells of the heterozygotic individual. In addition, resorption was recorded in chimeras estab-

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lished with colonies that were both homozygous at the Fu/HC locus [*i.e.*, AA *vs.* AA (4, 13)], which implied the involvement of additional genetic elements.

To further analyze this possibility, we established 121 pairs of chimeric partners by fusions of relatives from four generations within a pedigree, all homozygotes (AA line) at their Fu/HC haplotype (13). This was carried out by self- and defined-crosses done in the laboratory on two outbred founder colonies (each AB at the fusibility locus) which were taken from the field. We found that the resorption phenomenon within each generation of colonies is characterized by a linear hierarchy which is expressed by the existence of at least five intermediate hierarchial rungs (*i.e.*, colony A repeatedly resorbs colonies, B, C, D, E; colony B resorbs colonies C, D, E; and so on). Analysis of resorption hierarchies between generations revealed that mother colonies always resorbed their self-crossed offspring. Colonies low in the hierarchy within a specific generation reproducibly resorbed the self-crossed offspring of a superior kin. Chimeras between defined-crossed offspring and self-crossed offspring of different generations revealed nontransitive hierarchies (*i.e.*, colony A resorbs colony B; colony B resorbs colony C; but colony C resorbs colony A) in the resorption; these were correlated with the relative position of each colony in the linear hierarchy established within each generation (major results are schematically illustrated in Fig. 1).

In this essay I speculate on the genetic framework underlying the resorption phenomenon. I propose that allogeneic resorption in *Botryllus* is elicited and controlled by a polymorphic, ladder-like, tri-level hierarchial system of histocompatibility elements (Fig. 1; Table I). Hierarchy in the resorption phenomenon is best explained as being associated with the proportion of homozygosity to heterozygosity among the large numbers of polymorphic histocompatibility loci characterizing the partners within a chimera. Resorption (Fig. 1; Table I) is expressed morphologically in accordance with the following three assumptions:

Table I

A proposed ladder-like, tri-level, hierarchial organization of histocompatibility alleles in Botryllus schlosseri

Level no.	Controlling locus/loci*	Experimental protocol**	Major results***
1	Fu/HC	AX *vs* AA	Heterozygote > homozygote in most cases.
2	Re/HC	CAAs of self-crossing: generations II vs. III. All AA on Fu/HC.	Heterozygote > homozygote.
3	Re/HC	CAAs within generations II, III, IV, respectively. All AA on Fu/HC.	Linear hierarchy (at least 5 intermediate groups) in resorption within each generation. Heterozygote > homozygote?

* Fu/HC = fusibility-histocompatibility locus; Re/HC = resorption-histocompatibility locus or loci.

** CAA = colony allorecognition assay. Generation numbers refer to Rinkevich *et al.* (1993), Figs. 1, 2.

*** From Weissman *et al* (1990); Rinkevich *et al* (1993); Y. Saito (unpub.).

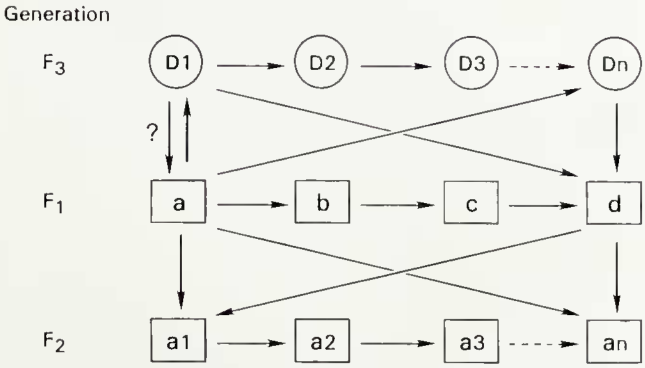

Figure 1. A schematic illustration of the hierarchies in the resorption phenomenon as revealed by a study of three successive generations of *Botryllus schlosseri* colonies, all AA on their Fu/HC locus (13). Squares refer to self-crossed offspring, circles are for the defined-crossed offspring. The arrows point to the inferior partner. Within each generation, colonies of the left side are the most dominant and those of the right side are the subordinates. The question mark refers to a possible additional route of resorption that was not completely verified. The letters a to d refer to specific colonies of the F_1 generation; a1 to an are the self-crossed offspring of colony a; and D1 to Dn are the defined-crossed F_3 colonies (13).

(1) *The levels.* The Fu/HC haplotype of tunicates is the first level of the theoretical multilevel hierarchial organization of histocompatibility alleles in *Botryllus*, and is the first to be morphologically expressed. This immunological locus encodes three types of cellular interactions: rejection, fusion, and resorption, all observable in Fu/HC disparate *Botryllus* colonies. The second level, which is controlled by specific resorption alleles (Fig. 1, Table I), operates only after the failure of resorption by the first level, is encoded by a different locus or loci, and has no effect on fusion-rejection processes. Similarly, the third level is expressed after lack of a resorption response from the second level.

(2) *Induction.* The resorption initiated by the second level of histocompatibility is elicited or stimulated by a trigger from the alleles of the first level. Moreover, these two levels are not genetically linked and are independently expressed. The same two attributes are relevant to the induction of the third level.

(3) *Re/HC alleles.* Colonies that are heterozygous with respect to the histocompatibility alleles in each of the three levels resorb homozygous colonies. At the first level, AA colonies on the tunicate Fu/HC will be absorbed by AX

colonies. But when both partners in a chimera are homozygotic (*i.e.*, AA *vs.* AA) or heterozygotic (*i.e.*, AB *vs.* AB; AB *vs.* BC) at the Fu/HC locus, the direction of the resorption cannot be determined by this level. At this point, the second level, controlled by the tunicate Re/HC (resorption-histocompatibility loci), is expressed, and the partner which is more heterozygous on this level will be the survivor. As a result, a mother colony will resorb its own self-crossed offspring, or the self-crossed offspring of a kin colony. I also assume that all histocompatibility alleles within each level are codominantly expressed. In any case, the relative state of homozygosity of these alleles within each level is reflected in the state of the resorption hierarchy.

Features of *Botryllus* histocompatibility that are homologous or analogous to vertebrate MHC and non-MHC recognition elements were also sought. An example is the assumption that Fu/HC and Re/HC heterozygotes will resorb Ru/HC and Re/HC homozygotes (Fig. 1; Table I). This is reminiscent of one type of rejection that occurs in the murine immune system; it is called the "hybrid resistance phenomenon" and is controlled by loci designated Hh-1 (14). In this phenomenon, irradiated F_1 heterozygotic mice that have been inoculated with syngeneic (homozygotic) bone marrow cells, reject these cells within a few days of grafting. The result is against the primary genetic rule of vertebrate transplantation: that an F_1 hybrid should not resist grafts from either of its parents.

Also intriguingly similar are the mouse minor lymphocyte stimulating (M1s) genes (15) which can stimulate proliferative responses of T-cells in mixed lymphocyte cultures that contain cells derived from strains identical at the MHC. M1s antigens are encoded by various mouse mammary tumor viruses that have been integrated into the murine germ line as DNA proviruses, but are inherited in a Mendelian fashion [reviewed in (16, 17)].

Below are some of the properties noted in the resorption phenomenon which are also observed in the M1s. As was suggested for the Fu/HC and the Re/HC loci (Fig. 1; Table I), several distinct M1s loci are scattered throughout different chromosomes (17, 18). Contrary to the usual bidirectional mixed lymphocyte reactions (MLRs) elicited by MHC disparaties, many of the M1s responses are unidirectional (18) and thus resemble the hierarchy of the tunicate resorption (13). M1s-specific MLRs (18), like responses elicited by other minor histocompatibility loci in the murine system (19, 20), have quantitative parameters in their expressions similar to the responses obtained in *Botryllus* resorption phenomena (4, 6, 7). M1s specific MLRs also show high polymorphism in the induction of clonal deletion *in vivo* and in T-cell activities *in vitro* (21). As in the *Botryllus* system, the *in vitro* T-cell proliferation that follows the mixing of naive M1s disparate cells, may be measured even without pre-immunizing the animal and *in vivo* challenge (16). Additionally, both the M1s (18) and the resorption (6) systems are characterized by some reciprocal response patterns. Neither the memory components of clonal expansion, nor long-term maintenance of heightened effector functions, were found in these two recognition responses (11, 16–18).

The above analysis of the resorption phenomenon has been studied solely by the assessment of *in vivo* responses of chimeras living in a laboratory setting. These studies (4–13) revealed, for the first time in invertebrates, a second morphologically expressed set of allorecognition responses that are, at least partly, unlinked to the first set of responses, the fusion-rejection processes (1–3). This speculative essay was meant to integrate the accumulated results of the last six years of work on *Botryllus* resorption phenomena into one plausible genetic framework—a hypothesis that will be challenged in further experimental tests. Of course the hypothesis is not perfect; remaining questions will be resolved in future experiments, which will include such diverse approaches as: studies with *Botryllus* inbred lines, *in vitro* experiments on blood cell lines (22), and biochemical and molecular initiatives.

Whether the tunicate resorption phenomenon evolved as a transplantation barrier unique to this group of organisms, or whether it contains an important evolutionary clue that will improve our general understanding of chordate immune systems, it will emerge as a unique example of an allorecognition system aimed at preserving clonal integrity.

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